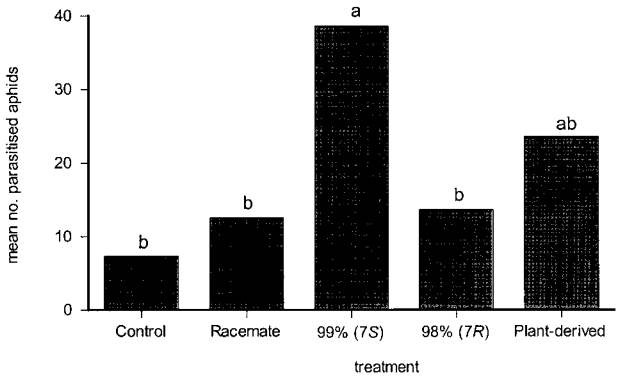


**Figure 1.** Parasitisation levels on aphid-invested plants baited with synthetic and plant-derived nepetalactone (**I**).

was no significant difference between the two nepetalactone treatments (Glinwood, RT, *et al.*, unpublished results).

The attractiveness of synthetic and plant-derived **I** to parasitoids was further examined in a semi-field experiment which employed aphid-infested trap plants. Analysis of the numbers of *P. volucre* mummies forming on the trap plants showed a significant effect of treatment ($P < 0.05$, 12 *df*, SED = 0.280, $F = 3.56$, Fig 1). Significantly more mummies formed on plants baited with 99% (7*S*)-**I** or plant-derived **I** than on unbaited control plants, whereas the number of mummies on plants baited with either (7*R*)-**I** or the 50% (7*S*)/(7*R*) racemate was not significantly greater than the control.

The manipulation of parasitoid behaviour was further examined by releasing plant-derived **I** in a winter-wheat field experiment. A comparison of pheromone-treated plots with control plots showed that parasitisation of aphids began earlier in the pheromone-treated plots. Thus it is demonstrated that the synthetic or plant-derived cyclopentanoid components of aphid sex pheromones have considerable potential for the manipulation of aphid parasitoids to enhance levels of parasitism and thereby induce aphid population control. The success of these experiments has led to the initiation of pilot systems for pheromone production from purified plant extracts, leading ultimately to the commercialisation of the aphid sex pheromones for the manipulation of beneficial insects.

## ACKNOWLEDGEMENTS

IACR-Rothamsted receives grant-aided support from the Biotechnology and Biological Sciences Research Council of the United Kingdom. Part of this work was supported by the United Kingdom Ministry of Agriculture, Fisheries and Food (MAFF), the Home-grown Cereals Authority, the Horticultural Development Council and the Processors and Growers Research Organisation under the MAFF-LINK scheme.

## REFERENCES

1 Dawson GW, Griffiths DC, Janes NF, Mudd A, Pickett JA, Wadhams LJ and Woodcock CM, Identification of an aphid sex pheromone. *Nature* (*London*) **325**:614–616 (1987).
2 Dawson GW, Pickett JA and Smiley DWM, The aphid sex pheromone cyclopentanoids: synthesis and the elucidation of structure and biosynthetic pathways. *Bioorg Med Chem* **4**:351–361 (1996).
3 Hardie J, Peace L, Pickett JA, Smiley DWM, Storer JR and Wadhams LJ, Sex pheromone stereochemistry and purity affect field catches of male aphids. *J Chem Ecol* **23**:2547–2554 (1997).
4 Hardie J, Nottingham SF, Powell W and Wadhams LJ, Synthetic aphid sex pheromone lures female parasitoids. *Entomol. Exp. Appl* **61**:97–99 (1991).

## Plant secondary metabolites regulating behaviour of zoospores of the phytopathogenic fungus *Aphanomyces cochlioides*

Satoshi Tahara,[1,2]* Masanori Mizutani,[1,2] Tomohiko Takayama[3] and Kaori Ohkawa[1]
[1] *Department of Applied Bioscience, Faculty of Agriculture, Hokkaido University, Kita-ku, Sapporo 060-8589, Japan*
[2] *CREST, Japan Science and Technology Corporation*

**Abstract: A number of compounds isolated from various plant species were tested for their ability to affect the mobility of zoospores of the fungus *Aphanomyces cochlioides* which causes root rot in spinach (*Spinacia oleracea*). Compounds may act as attractants, repellents or stimulants of zoosphore movement or they may halt movement by causing the spore to clump and settle. Bioassay revealed compounds with these methods of action, as well as some which acted directly on the fungus.**

**Keywords:** *Aphanomyces cochlioides*; zoospore attractant; repellent; stimulant; motility halting factors; spinach root rot

## 1 INTRODUCTION

*Aphanomyces cochlioides* Drechs is the fungus which causes spinach (*Spinacia oleracea*; Chenopodiaceae) root rot. Cochliophilin A (5-hydroxy-6,7-methylenedioxyflavone, Fig. 1, **1**) isolated from the roots of spinach, is a potent zoospore attractant. A second attractant, *N-trans*-feruloyl-4-*O*-methyldopamine (**2**) has been found in *Chenopodium album* L (Chenopodiaceae) which is also attacked by this pathogen, but which is not as susceptible as spinach. A third attractant, 5,4′-dihydroxy-3,3′-dimethoxy-6,7-methylendioxyflavone (**3**), has been isolated from the aerial parts of spinach. It is presumed that, prior to infection, the zoospores must be attracted to the roots of a compatible host plant which exude the host-specific signal substance(s). Alternatively, the roots of non-host plants may exude chemical signals indicating incompatibility with pathogens. This prompted a survey of plant metabolites which afforded unusual effects on the swimming behaviour of

* Correspondence to: Satoshi Tahara, Department of Applied Bioscience, Faculty of Agriculture, Hokkaido University, Kita-ku, Sapporo 060-8589, Japan.
(*Received 29 June 1998; accepted 30 September 1998*)

phytopathogenic fungal zoospores. Bioassay, using the zoospores of *A cochlioides*, was used to investigate the performance of some plant extracts.

## 2 METHODS

### 2.1 Bioassay

Particles of Chromosorb W AW (80–100 mesh) were used as a carrier of test compounds. One drop of an ethyl acetate solution of the test compound was dripped on to the particles on a watch-glass. Excess solution was immediately removed with a piece of filter paper, and the particles were then air-dried. One or two particles were carefully dipped into a zoospores suspension of *A cochlioides*, prepared as described previously,[1] in a small Petri dish on a microscope stage. The behaviour of zoospores around the particles was observed in comparison with that around reference particles treated with solvent alone. Zoospore movement around the particles containing non-active compounds was in a monotonous and straightforward fashion, and at a constant speed. In contrast, zoospores responded to the particles treated with active substances in one of the following manners. (i) *Attractant activity*: greater numbers of zoospores aggregated, massed and encysted around the particles, and their movement was in a complex zigzag or circular manner with increasing speed. (ii) *Repellent activity*: the zoospores were repelled from the particles, resulting in a circular zoospore-free zone. (iii) *Stimulant activity*: speed of zoospore movement near the particles increased without change in the population density. (iv) *Halting activity*: immediately after introduction of the particles, zoospore movement near the particles was halted and they finally sedimented at the bottom of the Petri dish.

## 3 RESULTS

### 3.1 Zoospore attractants

Some host-specific zoospore attractants have been reported in the literature, for example, indole-3-carboxaldehyde from *Brassica oleracea* L var *capitata* for *A raphani* Kendr zoospores,[2] prunetin from *Pisum sativum* L. for *A. euteiches* Drechs. zoospores,[3] and cochliophilin A (**1**) from *Spinacia oleracea* L[1] and an aromatic amide (**2**) from *Chenopodium album* L[4] both for *A cochlioides* zoospores. Interestingly, zoospores saturated with compound (**1**) can still show chemotaxis to the compound (**2**). Further survey of *A cochlioides* zoospore attractants resulted in isolation of another flavonoidal attractant, 5,4′-dihydroxy-3,3′-dimethoxy-6,7-methylenedioxyflavone-(**3**) from the aerial parts of spinach. Attractant activities (particle method, minimum concentration) were: **1**, $10^{-9}$–$10^{-10}$ M; **2**, $10^{-7}$–$10^{-8}$ M; **3**, $10^{-5}$–$10^{-6}$ M. Some other flavones related to **3** (**4**–**8**) were also subjected to the zoospore bioassay. However, they proved to be inactive in the particle test at 1 g litre$^{-1}$, whereas particles receiving **3** at 1–3 mg litre$^{-1}$ showed attractant activity. Compound **4** antagonised the attractant activity of **1** and **3**.

### 3.2 Halting factors for zoospore motility, a zoospore stimulant and a zoospore repellent

The ethyl-acetate-soluble constituents of *Portulaca oleracea* L roots showed a distinct and unusual effect on the swimming zoospores which were immobilised and settled on the bottom of the dish, the same phenomenon being observed around the fresh root segments of *P oleracea*. Further fractionation revealed that the activity to halt zoospore motility was caused by two factors, one a zoospore stimulant, *N-trans*-feruloyltyramine (**9**), and the other a repellent, thought to be a lisophosphatidic acid mono-methyl ester. Therefore, commercially available 1-oleoyl lisophosphatidic acid was derivatised to the corresponding mono-methyl ester (**10**), and the bioassay, using the particles treated with both **9** as 1 mg to 1 g litre$^{-1}$ solution and **10** as 10–100 mg litre$^{-1}$

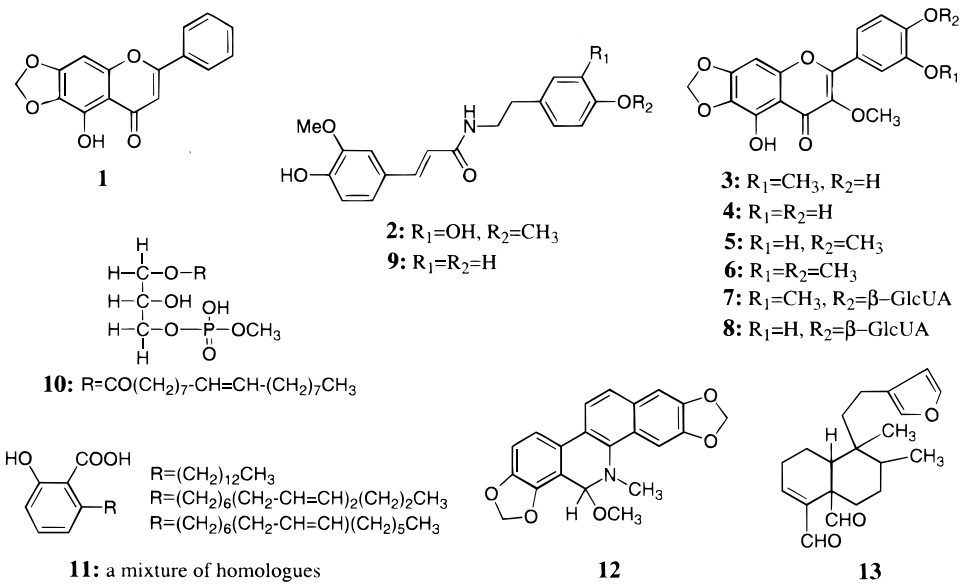


**Figure 1.** Plant secondary metabolites regulating behaviour of *Apahanomyces cochlioides* zoospores.

solution revealed that they regenerated the halted activity.

### 3.3 Zoosporicides

The methanol extract of unripe fruits of *Ginkgo biloba* L showed lytic activity toward the zoospores and the bioassay-guided fractionation resulted in isolation of a mixture of anacardic acid homologues (**11**) as an active principle.

### 3.4 Fungitoxins

Antifungal substances against *A. cochlioides* detected on agar plates by a paper disc method, were isolated from roots of *Chelidonium majus* L var *asiaticum* and *Solidago gigantea* (Aiton) var *leiophylla*, and identified as a sanguinarine alkaloid (**12**), inhibitory at 0.25 μg per disc) and a furan-containing diterpene (**13**, inhibitory at 2.5 μg per disc). The compounds are known to exist in *Fumaria indica* Pugsl seeds[5] and *Solidago serotina* (*S gigantea*),[6] respectively, and both inhibited growth of the fungus at 2.5 μg per disc).

## REFERENCES


1 Horio T, Kawabata Y, Takayama T, Tahara S, Kawabata J, Fukushi Y, Nishimura H and Mizutani J, A potent attractant of zoospores of *Aphanomyces cochlioides* isolated from its host plant, *Spinacia oleracea*. *Experientia* **48**:410–414 (1992).

2 Yokosawa R and Kuninaga S, *Aphanomyces raphani* zoospore attractant isolated from cabbage: Indole-3-aldehyde. *Ann Phytopath Soc Japan*, **45**:339–343 (1979).

3 Yokosawa R, Kuninaga S and Sekizaki H, *Aphanomyces euteiches* zoospore attractant isolated from pea root; Prunetin. *Ann Phytopath Soc Japan* **52**:809–816 (1986).

4 Horio T, Yoshida K, Kikuchi H, Kawabata J and Mizutani J, A phenolic amide from roots of *Chenopodium album*. *Phytochemistry* **33**:807–808 (1993).

5 Pandey VB, Ray AB and Dasgupta B, Minor alkaloids of *Fumaria indica* seeds. *Phytochemistry* **18**:695–696 (1979).

6 Anthonsen T, Henderson MS, Martin A, McCrindle R and Murray RDH, Furan-containing diterpenes from *Solidago serotina* Ait. *Acta Chem Scand* **22**:351–352 (1968).


## Role of the α subunit of nicotonic acetylcholine receptor in the selective action of imidacloprid


Kazuhiko Matsuda,[1]* Steven D Buckingham,[2] John C Freeman,[2] Michael D Squire,[2] Howard A Baylis[2] and David B Satelle[2]

[1] *Department of Agricultural Chemistry, Faculty of Agriculture, Kinki University, 3327-204 Nakamachi, Nara 631-8505, Japan*

[2] *The Babraham Institute Laboratory of Molecular Signalling, Department of Zoology, University of Cambridge, Downing Street, Cambridge CB2 3EJ, UK*

* Correspondence to: Kazahiko Matsuda, Department of Agricultural Chemistry, Faculty of Agriculture, Kinki University, 3327-204 Nakamachi, Nara 631-8505 Japan



Contract/grant sponsor: BBSRC
Contract/grant sponsor: Dupont
(*Received 28 June 1998; accepted 30 September 1998*)



**Abstract: Examination of agonist interactions of imidacloprid on recombinant chicken α4β2 and *Drosophila* SAD/Chicken β2 hybrid receptors, expressed in *Xenopus* oocytes by nuclear injection of the cDNAs, indicates that imidacloprid is a partial agonist. Replacement of the α4 subunit for the *Drosophila* SAD subunit lowered the imidacloprid $EC_{50}$ 37-fold, whereas $EC_{50}$s for other agonists increased 4-50 fold, suggesting that the α subunit contributes to the high affinity of insect nicotonic receptors for imidacloprid.**



**Keywords:** Imidacloprid; neonicotinoid; epibatidine; chicken α4β2 nicotinic receptor; *Drosophila* SAD subunit


## 1 INTRODUCTION

Chloronicotinyl insecticides and related nitromethylene heterocycles target insect nicotinic acetylcholine receptors (nAChRs).[1–3]. Electrophysiological and radioligand binding studies have shown these neonicotinoids to be more active against insect than against vertebrate nAChRs.[4,5] The molecular basis of the interactions of these insecticides with nAChRs remains to be determined.

nAChRs are heteropentamers; each molecule is composed of two α and three non-α subunits surrounding a central cation-selective ion channel that opens transiently in response to the binding of ACh or nicotinic agonists. From insect species, six nAChR subunits have been studied in detail, but apart from the αL1 subunit of *Locusta migratoria* L none of subunits from *Drosophila melanogaster* Meig is capable of forming functional receptors in any combinations tested so far in three heterologous expression systems (*Xenopus laevis* oocytes, HEK cells and *Drosophila* S2 cell lines.[6,7] Nevertheless, α subunits ALS, SAD and Dα3 of *Drosophila* are able to form functional receptors when co-expressed with the chicken β2 subunit (Reference 6, and E D Gundelfinger pers. comm.). Using this unique property we examined the sensitivity to imidacloprid of chicken α4β2 receptors and *Drosophila* SAD/chicken β2 hybrid receptors in an attempt to clarify the role of the α subunit in selectivity.

## 2 MATERIALS AND METHODS

Recombinant nAChRs were expressed in *Xenopus* oocytes by nuclear injection of cDNAs inserted into appropriate expression vectors, typically the pMT3 vector.[8] Briefly, the nucleus of each oocyte was injected with each cDNA (1 ng in 20 nl of distilled water). The injected oocytes were incubated at 17–18°C in standard oocyte saline (SOS): NaCl(100 mM) KCl(2 mM); $CaCl_2$(1.8 mM); $MgCl_2$(1 mM) and HEPES (5 mM; pH 7.5) supplemented with penicillin (100 units $ml^{-1}$), streptomycin (100 μg $ml^{-1}$), gentamycin (50 μg $ml^{-1}$) and sodium pyruvate (2.5 mM). Electrophysiological recordings were performed two to four days after incubation.